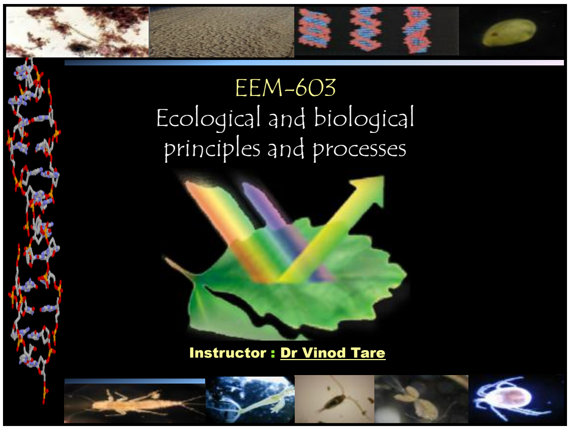


Biochemistry for Microbiology

- **Living Organisms**: Chemical machines made of chemical compounds and live by means of chemical reactions.
  - Thus understanding of chemistry is essential to understanding of living organisms.
- **Biochemistry:** Branch of chemistry, that deals specifically with chemistry in relation to life processes, such as chemical reactions involved in respiration and photosynthesis.

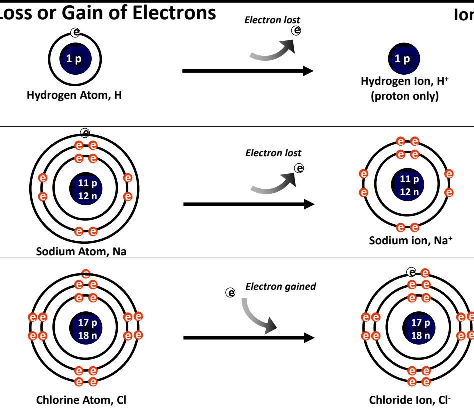
Living Organisms

Contains atoms and molecules as their most basic structural units

- The kind of interaction between atoms and molecules determines the fundamental qualities of compound such as solubility, acidity, etc.
- Microorganisms depend on soluble nutrients and are affected by their environment.
- Important chemical substances in living organisms are based on the element carbon, and include carbohydrates, lipids, proteins and nucleic acid.
- Biochemical processes (reactions) depend on special substances called enzymes, which greatly increase the speed at which a specific reaction occurs.
- By balancing the production and utilization of thousands of chemical, each microorganisms can adjust, and even contribute, to its surroundings.

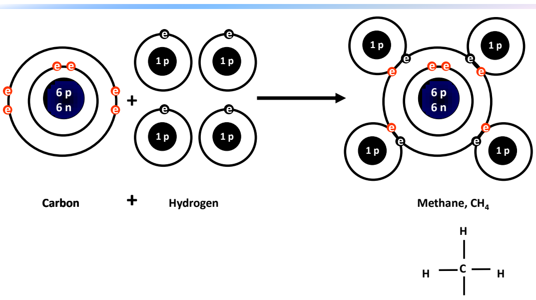
Loss or Gain of Electrons

Ionic Bond



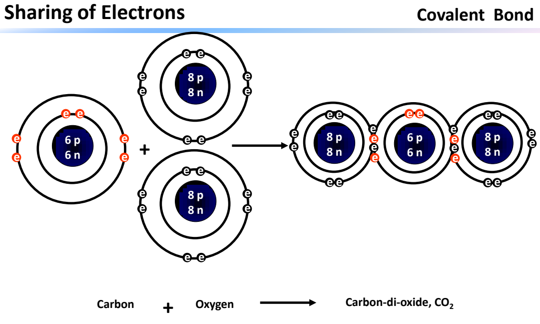
Sharing of Electrons

Covalent Bond

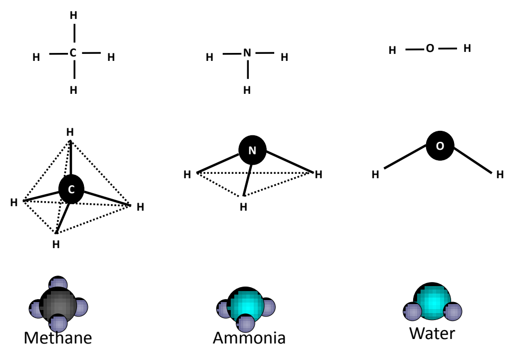


Sharing of Electrons

Covalent Bond

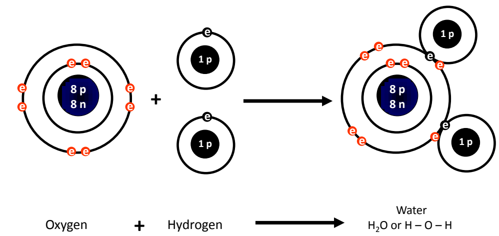


Geometric configuration of chemical bonds around carbon, nitrogen, and oxygen atom

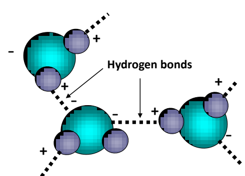


Sharing of Electrons – Unequal

Covalent Bond



Hydrogen Bond



Water molecules can be linked by hydrogen bonds. The small spheres are hydrogen atoms; the large spheres are oxygen atoms

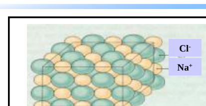
Importance of Water

- 80 to 90% of the weight of cell is actually water.
- Tends to resist heating/cooling.
- Fluid medium for most biochemical reactions.
- Direct participation in biochemical activities hydrolysis (splitting by water).
- Excellent solvent.

Solubility of Compounds in Water

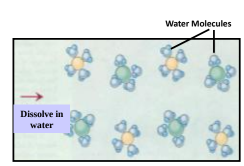
- The ability of ions to attract water molecules indicates that the ions are hydrophilic (water loving).
- Compounds that dissociate into ions are considered ionizable, and the presence of ionic groups confers water solubility on molecules.

Solubility in Water



Crystalline sodium chloride

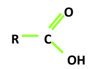
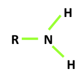
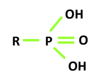
A sodium chloride crystal dissolves readily in water because the water molecules, which are electrically polar, become oriented to form hydration shells around the sodium ions and chloride ions and chloride ions. This helps to keep the ions separated from the another.



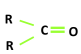
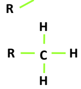
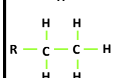
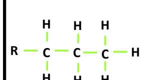
Solubility of Compounds in Water

- Polar & Ionizable Compounds → Soluble
- Non-polar Compounds → Insoluble (e.g. Oil, fats)
  - Soluble in non-polar solvents (no bonds between non-polar molecules, only aggregate)
- Amphipathic Compounds → both polar/ionized groups & non-polar region.
  - Soaps hydrophilic group inside and hydrophobic group outside
- Phospholipid → play an important role

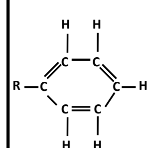
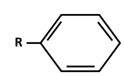
Functional Groups

Chemical Group*	Abbreviated Form	Name	Properties
	R — COOH	Carboxyl group (acidic)	Ionizes to R – COO^-
	R — NH_2	Amino group (basic)	Ionizes to R – NH_3^+
	R — PO_3H_2	Phosphate group (acidic)	Ionizes to R – PO_3^{2-}
R — OH	---------------	Hydroxyl group	Polar

Functional Groups

Chemical Group*	Abbreviated Form	Name	Properties
	R — CO — R	Carbonyl group (keto group)	Polar
	R — CH_3	Methyl group	Nonpolar
	R — CH_2 — CH_3	Ethyl group	Nonpolar
	R — CH_2 — CH_2 — CH_3	Propyl group	Nonpolar

Functional Groups

Chemical Group*	Abbreviated Form	Name	Properties
		Phenyl group	Nonpolar

Role of Acid, Bases, pH Buffer

- Water ionizes poorly one liter 55.55 mol of water but only 10^{-7} mol is ionized out of 555,500,000 molecules only one molecule is separated to H & OH.

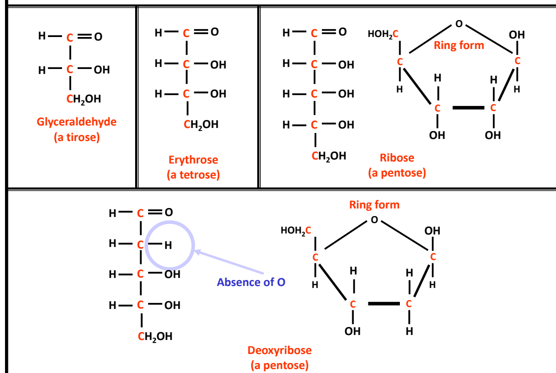
Important Biological Compounds

- Cell of all living organisms, from microbes to humans, are composed of chemical compounds.
- In-organics as well as organic compounds, but organic compounds have the most biological significance.
- Most of organic compounds can be grouped into one of four main categories
  - Carbohydrates,
  - Lipids
  - Proteins and
  - Nucleic acids

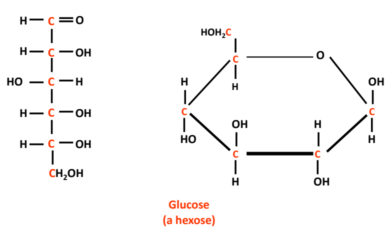
Carbohydrates

- Sugars and starches are carbohydrates
- Primary source of energy in cell
- Some carbohydrates are also found in cell walls, while others serve as food storage and act as building blocks for proteins, lipids and nucleic acids.
- General formula $(CH_2O)_n$ (any whole number)
- They can be quiet simple in structure or contain a large number of molecules arranged in complex ways.
- Simplest carbohydrates are Monosaccharides or (simplest sugars)
- Large number of monsaccharrides linked together are referred to as polysaccharides → As in a molecule of starch

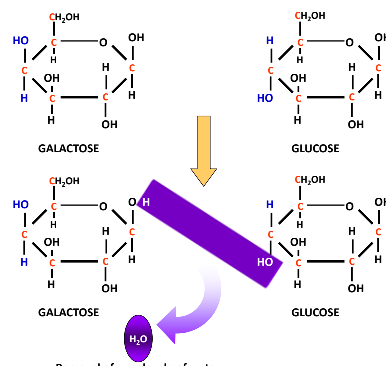
Some examples of monosaccharides, or simple sugars



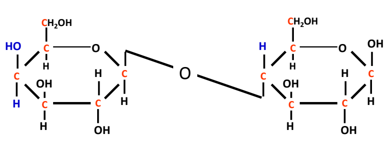
Some examples of monosaccharides, or simple sugars



Di- / Poly- saccharides

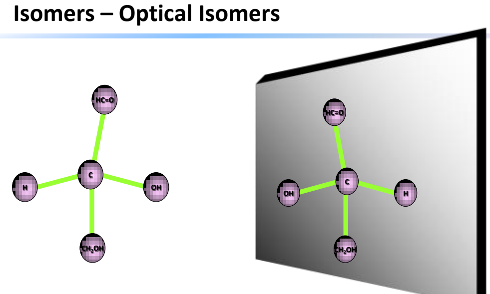


Di- / Poly- saccharides



Lactose
(a disaccharide)

Isomers – Optical Isomers



Glyceraldehyde has an asymmetric carbon atom and thus can exist as two optical isomers, the D and L forms, which are mirror images of each other.

Lipids

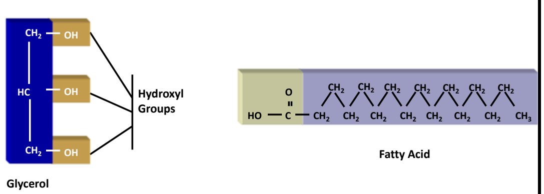
- Organic substances soluble in non-polar solvents such as acetone, chloroform, ether or benzene.
- Most lipids are insoluble in water
- Composed mainly of H and C atoms and less of other elements such as O, N and P
- There are three major categories of lipids (biologically important) based on differences in structure: fats, phospholipids, and sterols.

Fats

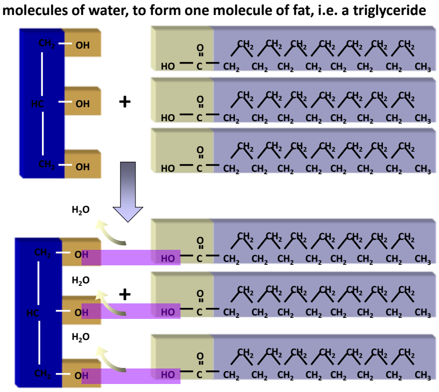
- Fats are simple lipids made of two kinds of building blocks: glycerol and fatty acids
- Fats are formed when three molecules of fatty acids are attached by an enzymes to one molecule of glycerol
- Hence called as triglycerides

Fats

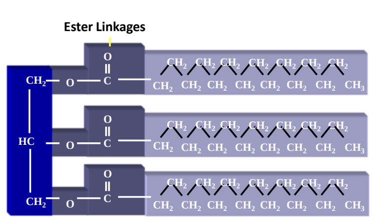
Glycerol molecules and fatty acid molecules are the building blocks of fats.



Three fatty acid molecules are linked to one molecule of glycerol by removing three molecules of water, to form one molecule of fat, i.e. a triglyceride



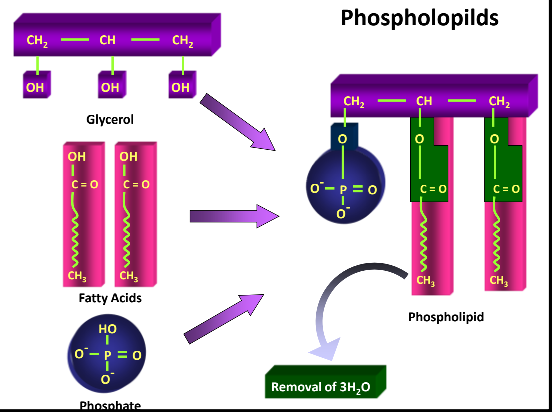
Fats



Phospholopilds

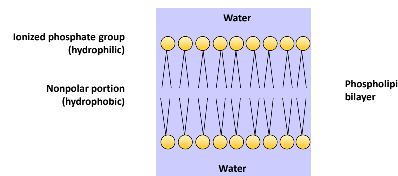
- Phospholipids are complex lipids, are important component of cell membranes. (for e.g. 22 million phospholipid molecules are found in *Echerichia coli* (a single cell bacterium)
- Only two molecules of fatty acids are linked to a molecule of glycerol
- A phosphate group is linked to the glycerol

Phospholopilds



Phospholopilds

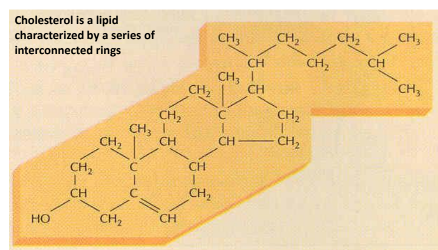
When placed in water, the amphipathic phospholipid molecules form a bilayer, with the non polar hydrocarbon chains of the fatty acid facing inward and the negatively charged phosphate groups facing outward.



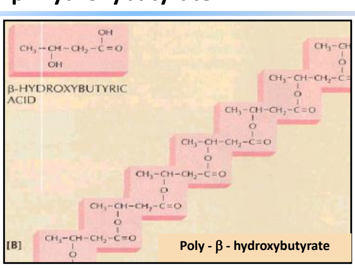
Sterols

- Highly non-polar, consist mainly of several interconnected rings made of carbon atoms.
- Animals use them to synthesize vitamin D and steroid hormones.
- Found in membranes of eucaryotic cells and a few bacteria.
- The compound cholesterol, a normal component of some membranes, is a member of this group of lipids i.e. sterol.
- Several anti fungal drugs combine with sterols in membrane of fungus cells, eventually killing the cells.

Cholesterol



Poly- β- hydroxybutyrate



Poly- β- hydroxybutyrate is a chain of many molecules of β hydroxybutyric acid linked together by removal of water molecules, only a small portion of the entire chain is shown

Other lipids

- Lipids in chlorophyll, those in cell walls of the bacterium that causes tuberculosis, and those that provide the red and yellow pigments of same microorganisms
- A lipid called PHB (poly-B-hydroxybutyrate) occurs only in certain bacteria as a reserve source of carbon and energy
- Insoluble in water and even in some non-polar solvents, such as alcohol and ether
- Soluble in hot chloroform.

Proteins

- In terms of weight, proteins surpass lipids and carbohydrate in a cell.
- Multiple functions: some may be enzymes, the catalytic agents that control all biochemical processes; others may be part of cell structures, such as flagella; or they may control nutrient transport through membranes; Toxins produced by cells are proteins.
- Composed of amino acids (building blocks)

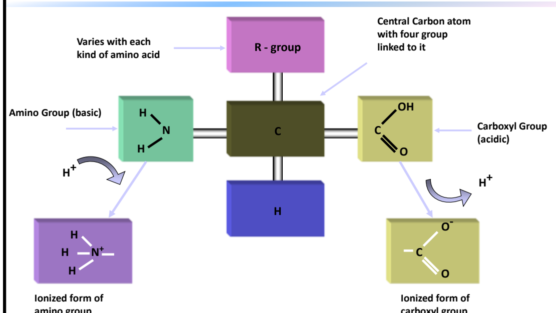
Amino Acids

The 20+ kinds of amino acids from which proteins are formed; all have one part of their structure in common but differ in their R groups. The central carbon is asymmetric if all four groups linked to it differ from one another, as is the case for most amino acids.

A standard abbreviation for the name of each amino acid is used.

General Structure of an Amino Acid

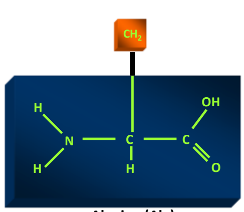
The amino group is basic and can take up a hydrogen ion to become positively charged, whereas a carboxyl group is acidic and can liberate a hydrogen ion to become negatively charged.

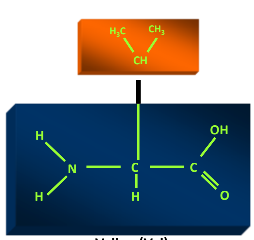


Amino Acids

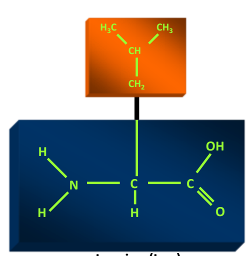
- An amino group, $-NH_2$, can take up H ion, basic group.
- A carbonyl group, -COOH, can release H ion; acidic group.
- A hydrogen atom; and
- An "R" group which varies with each kind of amino acid.
- Amino acids (20 in number) consist of four chemical groups attached to carbon atom. Several amino acids linked together in a chain from a protein molecule.
- In most amino acids, Carbon atom is asymmetric, since the four group differ from one another.
- The only exception is glycine – two groups are H atoms .
- Because of asymmetric carbon atom, an amino acid can exist as either of two optical isomer, mostly L isomer in living organisms, D isomers are rare, although certain ones do occur in cell walls of bacteria.

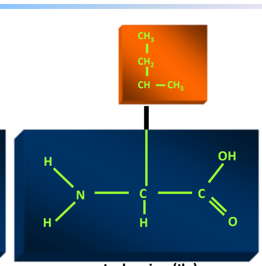
Amino Acids


Alanine (Ala)

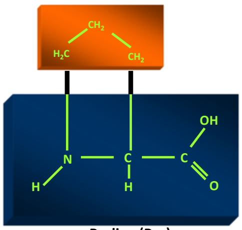

Valine (Val)

Amino Acids

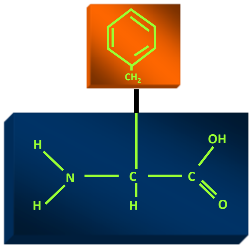

Leucine (Leu)


Isoleucine (Ile)

Amino Acids

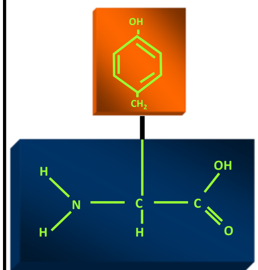


Proline (Pro)

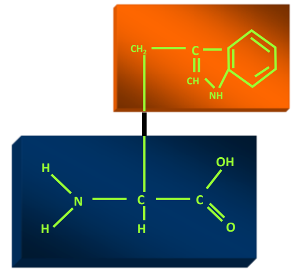


Phenylalanine (Phe)

Amino Acids

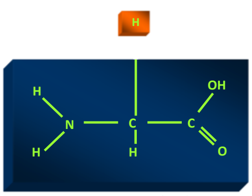


Tyrosine (Tyr)

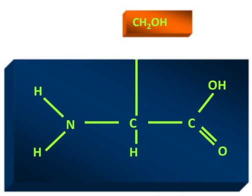


Tryptophan (Trp)

Amino Acids

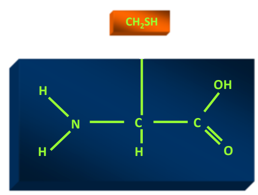


Glycine (Gly)

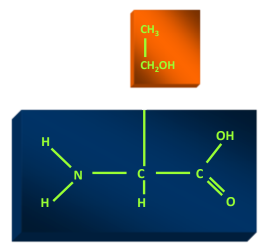


Serine (Ser)

Amino Acids

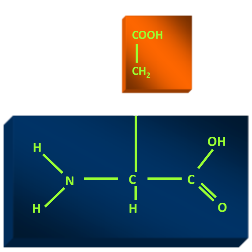


Cysteine (Cys)

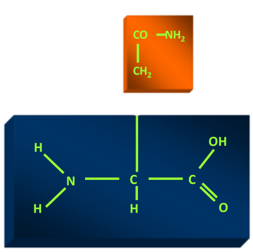


Threonine (Thr)

Amino Acids

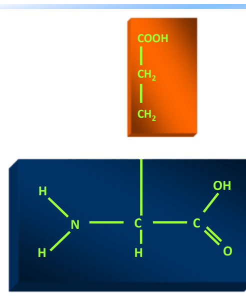


Aspartic acid (Asp)

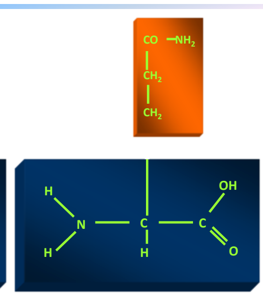


Asparagine (Asn)

Amino Acids

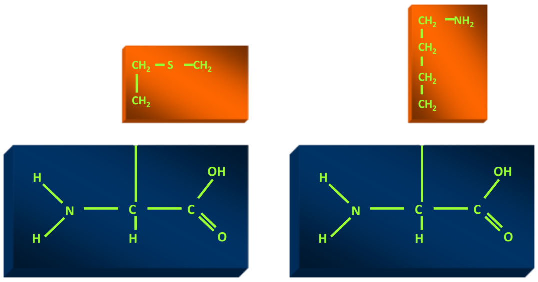


Glutamic acid (Glu)



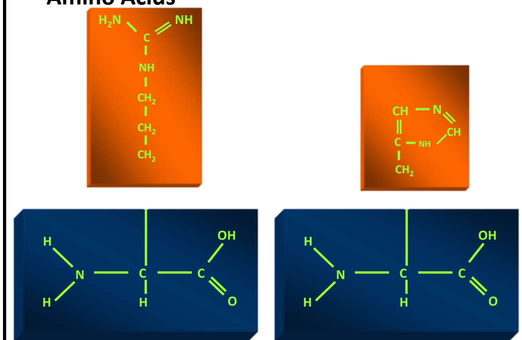
Asparagine (Asn)

Amino Acids



Methionine (Met)   Lysine (Lys)

Amino Acids



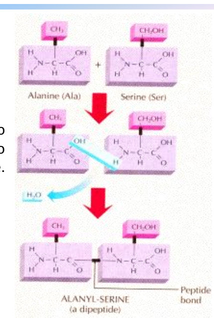
Arginine (Arg)   Histidine (His)

Peptide Bond

- Formed by removal of water molecule, tie together amino acids to form a long chain, called a polypeptide chain.
- Proteins consist of one or more of these polypeptide chains, which may change in length from fewer than 100 amino acids to more than 1000.

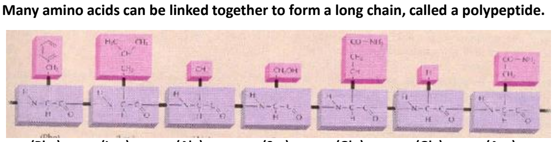
Di-peptide

By removing a molecule of water, two amino acids can be linked together to form a dipeptide.



Polypeptide

Many amino acids can be linked together to form a long chain, called a polypeptide.



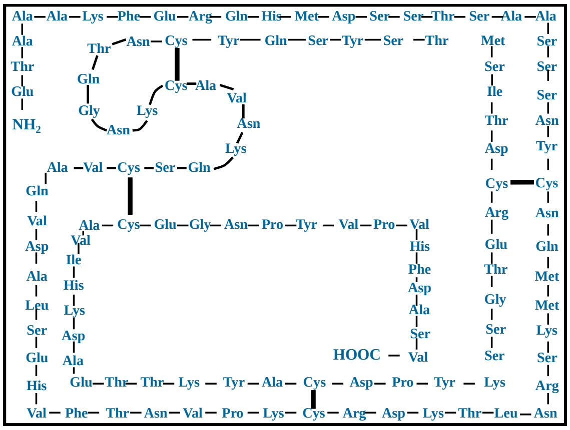
(Phe) (Leu) (Ala) (Ser) (Gln) (Gly) (Asn)

Portion of polypeptide chain

Levels of Protein Structure

A living cell contain 1000 or more different kinds of proteins, and each kind has its own unique sequence of amino acids. The amino acids sequence is called the primary structure of the protein.

(for e.g. ribonuclease contains 124 amino acids in a specific sequence).

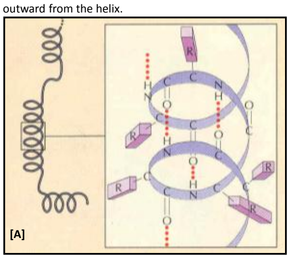


Protein Structure

- **Secondary structure:** A polypeptide chain can fold into a specific shape, much like a ribbon. Some portions of chain may form a coil, while others may form a side-by-side arrangements or other configurations. These forms constitute the secondary structure and are due to H bonding between polar –C=O and –NH groups along the chain.
- **Tertiary structure:** Overall folding of molecules into a specific shape, caused by interaction between different types of polypeptide chains. For instance, di sulfide bridges or bonds between sulphur ions contribute to the tertiary structure by connecting cysteine molecules located in different regions of the polypeptide chain.
- **Quaternary Structure:** Some proteins contain two or more polypeptide chains for their proper activity. This combination of polypeptide chains constitutes the quaternary structure. For example, the blood protein hemoglobin contains four polypeptide chains.

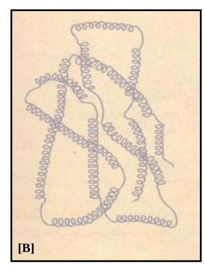
Protein Structure

[A] Secondary structure of a protein. Portions of the polypeptide chain from an alpha helix due to hydrogen bonding (•••••) between –C=O and –NH groups of the peptide bonds. (for simplicity, only the hydrogen atoms actually involved in the hydrogen bonding are shown.) The R Groups of the amino acids in the chain project outward from the helix.



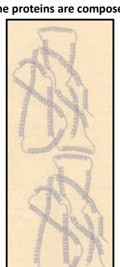
Protein Structure

[B] The tertiary structure of a protein is determined by interactions between portions of the chain



Protein Structure

[C] Quaternary structure of a protein. The protein shown here is composed of two identical polypeptide chains, but some proteins are composed of several different kinds of chains.



Nucleic Acids

- Discovery of chemical substance that carries genetic information of cells was one of the most exciting findings of the twentieth century (by American microbiologists)
- DNA & another substance, first found in nuclei of cell, ribonucleic acids (RNA), are called nucleic acids.
- DNA (deoxyribonucleic acid) is the substance responsible for the inheritable characteristics of living organisms.
- DNA is the substance that contains the hereditary information of a cell, whereas RNA is usually involved in deciphering the hereditary information in DNA and carrying out its instructions.

DNA

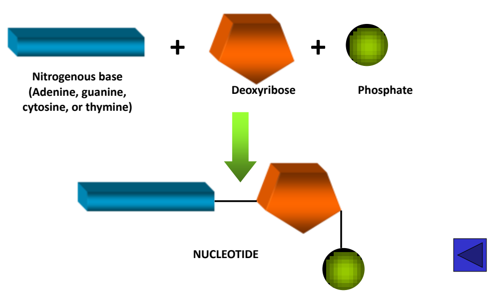
- Longest molecules in living cells (=1000 times longer than the cell itself)
- Fits into the cell because it is twisted into a highly compact form.
- A single molecule of DNA contains a vast library of hereditary information.
- But it has relatively simplest chemical structure.

DNA

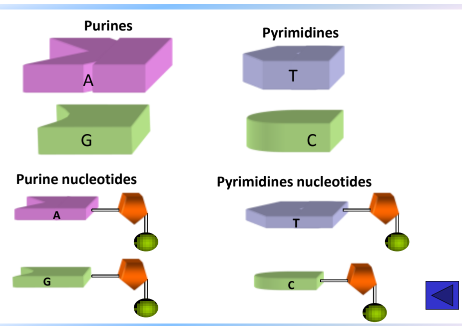
Composition:

- A DNA molecule is composed of molecules called nucleotides.
- Each nucleotides consists of three parts
  1. One molecule of a class of nitrogen containing compounds called nitrogenous bases.
  2. One molecule of the pentose sugar deoxyribose.
  3. One phosphate group.
- By using energy from food sources, a cell links these three parts to form a nucleotide.
- Four kinds (two groups) of nitrogen bases occur in DNA
  1. Adenine and Guanine → Called Purines
  2. Cytosine and Thymine → Called Pyrimidines.
- A cell puts together thousands of nucleotides to form a single strand of DNA. Two things are interesting about this strand:
  1. Each phosphate is attached to two deoxyribose. And,
  2. Phosphate and deoxyribose alternate to form a "backbone" from which project the purines and pyrmidines.

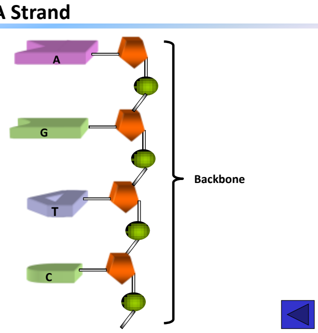
Nucleotide



Nucleotides



Portion of DNA Strand



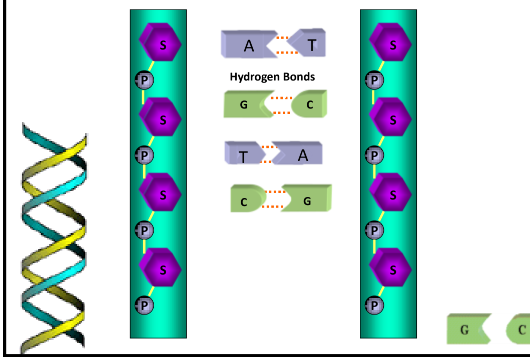
Double Stranded DNA

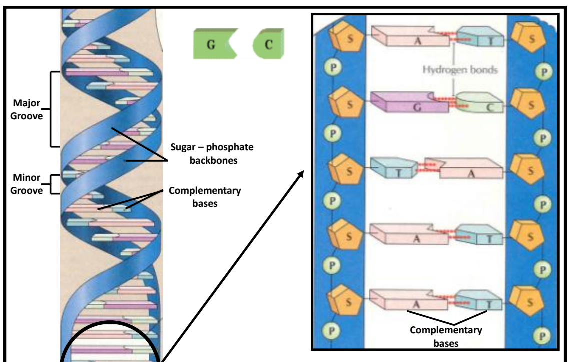
- Finally, two strands are cross-linked by means of the projecting purines and pyrimidne bases to form double stranded DNA.
  - Hydrogen bonds link the bases on one chain with those on the other chain.
  - The two bases attached in this manner are complimentary and called "complimentary base pair".
  - Only two kinds of complimentary base pairs are found in DNA.
  - Thus ratio of A to T or G to C is always 1:1 in double stranded DNA.
  - The complimentary of the purines and pyrimidines means that the sequence of bases on one strand dictates the sequence on the other.
  - This is of critical importance in the synthesis of new strands of DNA during cell division, because it is the sequence of bases in DNA that represents the hereditary information of cell. There is a different sequence for each species of living organism.

Double Stranded DNA

- In the double-stranded DNA molecule, the two strands are not straight, but are wound around each other to form a double helix (two strands in a double helix are held by H bonds between the complementary bases).

In double-stranded DNA, the two strands were wound about each other in the form of a double helix and are held together by hydrogen bonds between complementry purine and pyrimidine bases. P, Phosphate; S, the sugar deoxyribose





In double-stranded DNA, the two strands are wound about each other in the form of a double helix and are held together by hydrogen bonds between complementary purine and pyrimidine bases. P: phosphate; S: the sugar deoxyribose.

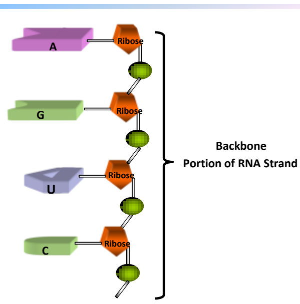
RNA

- RNA is also composed of a chain of nucleotides, but it differs from DNA in certain respects.

1. Sugar in RNA is ribose and not deoxyribose.
2. RNA contains pyrimidine called uracil instead of thymine.
3. Unlike double stranded DNA, RNA is single stranded. This means that there is no complementary second strand paired with it. Thus the ratio of A to U or G to C can vary and is not necessarily 1:1.

RNA

The structure of RNA. RNA differs from DNA by having the sugar ribose instead of deoxyribose, and the pyrimidine uracil (U) instead of thymine. The other three bases (A, G, and C occur in both RN and DNA. Unlike DNA, RNA is single standarded.



Enzymes

- Serve as catalytic agents.
- Specific to chemical reactions.
- Some are capable of increasing the rate of a chemical reaction millions of times over that of spontaneous reaction.
- Sensitive to their surroundings.
- Can be inhibited in various ways (Enzyme inhibition) → (H_2 & O_2 will make long time to form water, finely divided into powder of Platinum can act as a catalyst to form water instantaneously)
- Unlike inorganic catalyst, enzymes are organic substances produced by living cells.

Enzymes

- Until recently all enzymes were thought to be proteins but recently (in 1989 Sidney Altman of Yale University and Thomas Cech of the university of Colorado received Noble prize in chemistry) it has been discovered that RNA can also catalyze certain reactions in cell. This discovery has revolutionized the ideas held by biochemists about the origin and nature of enzymes.
- Some enzymes are pure proteins, but many consist of a protein combined with a much smaller non-protein molecule (called coenzyme), which assists the protein portion (called the apoenzyme), by accepting or donating atoms when needed.

Apoenzyme + Coenzyme = Holoenzyme

Vitamins

- May be coenzyme or principle components of a particular enzyme.
- Are organic substances that occur naturally in very small amounts but are essential for all cells.
- The vitamins that an organism cannot synthesize, must be supplied in the diet.
- Inorganic coenzymes (Mg, Zn, etc.) are called as cofactors
- Sometimes both a cofactor and a coenzyme are required before an enzyme is able to act as catalyst.

Some Coenzymes and Their Constituent Vitamins

Coenzymes	Vitamin
Coenzyme A (CoA)	Pantothenic acid
Cocarboxylase (thiamine pyrophosphate, TPP)	Thiamine (B_1)
Flavin adenine dinucleotide (FAD)	Riboflavin (B_2)
Nicotinamide adenine dinucleotide (NAD) and nicotiamide adenine dinucleotide phosphate (NADP)	Niacin (nicotinic acid)
Pyridoxal phosphate	Pyridoxal (B_6)
Tetrahydrofolic acid (THF)	Folic acid

Enzymes and Their Classification

- Although there are thousands of kinds of enzymes, they can be grouped into six major classes.
- The name of any enzyme always has the suffix -ase and is usually based on the particular chemical reaction it catalyzes. For example an enzyme that removes hydrogen atoms from Lactic acid are called as ***lactic acid dehydrogenase.***

Major Classes of Enzymes

Class No	Class Name	Catalytic Reaction	Example of Enzyme and the Reaction it Catalyzes
1	Oxidioreductases	Electron-transfer reactions (transfer of electrons or hydrogen atoms from one compound to another)	Alcohol dehydrogenase: Ethyl Alcohol + NAD↔ acetaldehyde + $NADH_2$
2	Transferases	Transfer of functional groups (such as phosphate groups, amino groups, methyl groups)	Hexokinase: D-Hexose + ATP ↔ D-Hexose-6-phosphate
3	Hydrolyses	Hydrolyses reactions (addition of water molecule to broke a chemical bond)	Lipase: Triglyceride + H_2O ↔ diglyceride + a fatty acid
4	Lyases	Addition to double bonds in a molecule as well as non hydrolytic removal of chemical groups	Pyruvate decarboxylase: Pyruvate ↔ acetaldehyde + CO_2

Major classes of Enzymes contd…..

Major Classes of Enzymes

Class No	Class Name	Catalytic Reaction	Example of Enzyme and the Reaction it Catalyzes
5	Isomerases	Isomerization reactions (in which one compound is changed into another having the same number of kinds of atoms but differing in molecular structure	Triphosphate isomerase: D-Glyceraldehyde-3-phosphate ↔ Dihydroxyacetone phosphate
6	Ligases	Formation of bonds with cleavage or breakage of ATP (adenosine tri phosphate)	Acetyl-coenzyme A synthetase ATP+acetate+coenzyem A ↔ AMP+pyrophosphate+acet--ylcoenzyme A

Enzyme Substrate Complex:

E + S ↔ ES → E + P

- Typically one enzyme molecule can catalyze the conversion of 10 to 1000 molecules of substrate to products in one second.
- Catalyzed reactions may be several thousands to billion times faster than same reactions without enzymes.
- If enzymes were absent, the break down of protein in human digestive processes would take more than 50 yrs instead of few hours.

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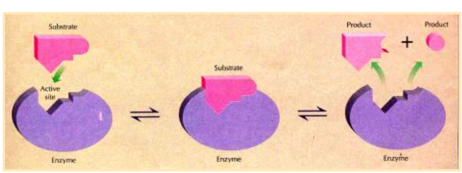
Enzyme-Substrate Reaction

The substrate combines with the enzyme at the active site, which is configured to fit the substrate molecule. In the case illustrated, the chemical groupings of the enzyme-bound substrate are strained so that cleavage of the substrate results . The cleavage products are released form the enzyme, and the enzyme is free to combine with more substrate and repeat the process.



Enzyme Specificity

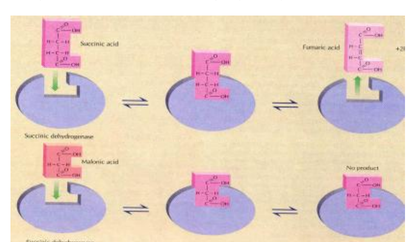
- A single enzyme may react with one substrate or at the most with a group of closely related substrates.
- Each enzyme causes a one step change.
- Most biological processes thus require a form of cooperation among groups of enzymes, rather like a relay team running a long race.
- Enzyme specificty is based to a great extent on the three dimensional structure of the active site on the enzyme molecule.
- Specifically related to L & D isomer (left hand glove to right hand glove)

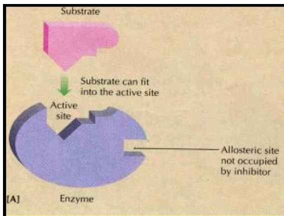
Enzyme Inhibition
By physical or chemical conditions

- Competitive inhibition
- Non competitive inhibition
- Feedback inhibition → Allosteric inhibition: a non competitive inhibition in which an inhibitor (in this case the product molecule) binds to the enzyme at some place other than the active site. This distorts the active site so that the substrate no longer fits into it.
- Lowers enzyme activity

Competitive Inhibition

Competitive inhibition (schematic diagram) of the enzyme succinic dehydrogenase by malonic acid. Malonic acid has a structure that is similar to that of the substrate, succinic acid, allowing it to compete with the substrate for attachment to the active site on the enzyme surface. If malonic acid occupies the site, further enzyme activity is blocked, as malonic acid is not changed by this enzyme.

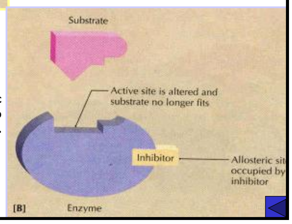




Allosteric inhibition of an enzyme.

[A] An allosteric site is located at a region on the enzyme other than the active site. When the allosteric site is unoccupied, the substrate for the enzyme can fit into the active site.

[B] When a specific inhibitor occupies the allosteric site, the active site is distorted and substrate no longer fits.



END OF PART 3